

TRANSMITTAL LETTER TO THE UNITED STATES

200204US0PCT

DESIGNATED/ELECTED OFFICE (DO/EO/US)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

CONCERNING A FILING UNDER 35 U.S.C. 371

09/701572

INTERNATIONAL APPLICATION NO.
PCT/FR99/01342INTERNATIONAL FILING DATE
08 JUNE 1999PRIORITY DATE CLAIMED
08 JUNE 1998

TITLE OF INVENTION

PLANT PROTEIN WITH REPEATED WD40 MOTIFS, NUCLEIC ACID CODING FOR SAID PROTEIN, AND
USES THEREOF

APPLICANT(S) FOR DO/EO/US

Eva KONDOROSI, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
A **SECOND** or **SUBSEQUENT** preliminary amendment.
16. ☐ A substitute specification.
17. ☐ A change of power of attorney and/or address letter.
18. ☐ Certificate of Mailing by Express Mail
19. ☒ Other items or information:

Request for Consideration of Documents Cited in International Search Report

Notice of Priority

Drawings (8 sheets)

PCT/IB/304

PCT/IB/308

U.S. APPLICATION NO. 097701372	INTERNATIONAL APPLICATION NO. PCT/FR99/01342	ATTORNEY'S DOCKET NUMBER 200204USOPCT
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20. The following fees are submitted:				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :					
<input checked="" type="checkbox"/>	Search Report has been prepared by the EPO or JPO	\$860.00			
<input type="checkbox"/>	International preliminary examination fee paid to USPTO (37 CFR 1.482)	\$690.00			
<input type="checkbox"/>	No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	\$710.00			
<input type="checkbox"/>	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO	\$1000.00			
<input type="checkbox"/>	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	\$100.00			
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$ 860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)). <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30				130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =		x \$18.00	0.00	
Independent claims	- 3 =		x \$80.00	0.00	
Multiple Dependent Claims (check if applicable). <input type="checkbox"/>				0.00	
TOTAL OF ABOVE CALCULATIONS =				\$ 990.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable). <input type="checkbox"/>				0.00	
SUBTOTAL =				\$ 990.00	
Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). <input type="checkbox"/> 20 <input type="checkbox"/> 30				0.00	
TOTAL NATIONAL FEE =				\$ 990.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). <input type="checkbox"/>				0.00	
TOTAL FEES ENCLOSED =				\$ 990.00	
				Amount to be:	\$
				refunded	\$
				charged	\$


☒ A check in the amount of \$ 990.00 to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:



22850

Surinder Sachar
Registration No. 34,423

Surinder Sachar

SIGNATURE

Norman F. Oblon

NAME

24,618

REGISTRATION NUMBER

Dec. 8 2000

DATE

200204US-369-917-0 PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :
EVA KONDOROSI ET AL : ATTN: APPLICATION DIVISION
SERIAL NO: 09/701,572
FILED: DECEMBER 8, 2000 :
FOR: PLANT PROTEIN WITH REPEATED
WD40 MOTIFS, NUCLEIC ACID
CODING FOR SAID PROTEIN, AND
USES THEREOF

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE CLAIMS

Claim 8, lines 1-2, replace "either of Claims 1 and 2, or a nucleic acid sequence according to Claim 3" with --Claim 1--.

REMARKS

Claims 1-11 are active in the present application. The claims are amended to remove multiple dependencies. No new matter is added. An action on the merits and allowance of the claims is solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Norman F. Oblon
Attorney of Record
Registration No. 24,618

Daniel J. Pereira, Ph.D.
Registration No. 45,518



22850

(703) 413-3000
Fax #: (703)413-2220

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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :
EVA KONDOROSI ET AL : ATTN: BOX SEQUENCE
SERIAL NO: 09/701,572 :
FILED: DECEMBER 08, 2000 :
FOR: PLANT PROTEIN WITH REPEATED
WD40 MOTIFS, NUCLEIC ACID CODING
FOR SAID PROTEIN, AND USES THEREOF

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Responsive to the Office Communication dated May 4, 2001, Applicants submit herewith a substitute Sequence Listing and a corresponding computer-readable Sequence Listing. Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Please amend the specification as shown in the marked-up copy to read as follows:

Page 5, lines 1-10 replace the text in its entirety with the following:

Figures 1B and 1C represent alignment, carried out using the "PRETTYBOX" software, of *Meedicago sativa* CCS52 (MsCCS52) sequence (SEQ IS NO:2) and the *Drosophila* FZY and FZR (DmFZY and DmFZR) sequence (SEQ ID NOS:7 and 8), of the *Saccharomyces cerevisiae* HCT1 (SchCT1) sequence (SEQ ID NO:9), the

Schizosaccharomyces pombe SRW1 (SpSRW1) sequence (SEQ ID NO:10), the *Arabidopsis thaliana* FZY (AtFZY) sequence (SEQ ID NO:11) and the 2 *Arabidopsis thaliana* polypeptides (AtCCS52a = peptide deduced from AL31018 (SEQ ID NO:12), and AtCCS52B = peptide deduced from AB005230 (SEQ ID NO:13).

Page 18, lines 11-13, replace the text in its entirety with the following:

P55BL : TTTGGGGGTTGATGATTGTG SEQ ID NO:3

P55CL :CTCTCTACCGTTCTATCTCTTGGGA SEQ ID NO:4

P5CR :GGTAAAGATGCTACTTTGGTGGTGT SEQ ID NO:5

Page 20, lines 24-29, replace the text in its entirety with the following:

Construction of pISV-BMCS: pISV2301 is digested with HindIII and SstI in order to eliminate the sequence of the 2X35S-AMV promoter, which is replaced by the following double-standed BMCS oligonucleotide:

AGCTTCCCGGGGGAGCTCTAGACTCGAGCAGCT

AGGCCCTCGAGATCTGAGCTCG (SEQ ID NO:6).

Page 23 (Abstract), after the last line, beginning on a new page replace the original Sequence Listing with the substitute Sequence Listing appended herewith.

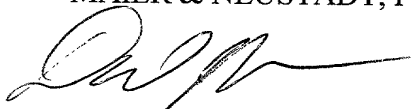
REMARKS

Applicants have now submitted a substitute Sequence Listing and a corresponding computer-readable Sequence Listing. Sequence Identifiers (SEQ ID NO:) have been added to the specification. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the substitute Sequence Listing. Support for all of the sequences listed in the substitute Sequence Listing is found in the present application as originally filed. No new matter is believed to have been introduced by the submission of the substitute Sequence Listing and the corresponding computer-readable Sequence Listing.

Applicants submit that the present application is ready for examination on the merits. Early notification of such is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Norman F. Oblon
Attorney of Record
Registration No. 24,618

Daniel J. Pereira, Ph.D.
Registration No. 45,518



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(703) 413-3000

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Marked-Up Copy
Serial No: 09/710,572
Amendment Filed on:
July 5, 2001

IN THE SPECIFICATION

Please amend the specification as follows:

Page 5, lines 1-10 replace the text in its entirety with the following:

Figures 1B and 1C represent alignment, carried out using the "PRETTYBOX" software, of *Medicago sativa* CCS52 (MsCCS52) sequence (SEQ IS NO:2) and the *Drosophila* FZY and FZR (DmFZY and DmFZR) sequence (SEQ ID NOS:7 and 8), of the *Saccharomyces cerevisiae* HCT1 (SchCT1) sequence (SEQ ID NO:9), the *Schizosaccharomyces pombe* SRW1 (SpSRW1) sequence (SEQ ID NO:10), the *Arabidopsis thaliana* FZY (AtFZY) sequence (SEQ ID NO:11) and the 2 *Arabidopsis thaliana* polypeptides (AtCCS52a = peptide deduced from AL31018 (SEQ ID NO:12), and AtCCS52B = peptide deduced from AB005230 (SEQ ID NO:13).

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P55CL :CTCTCTACCGTTCTATCTCTTGGGA SEQ ID NO:4
P5CR :GGTAAAGATGCTACTTTGGTGGTGT SEQ ID NO:5

Page 20, lines 24-29, replace the text in its entirety with the following:

Construction of pISV-BMCS: pISV2301 is digested with HindIII and SstI in order to eliminate the sequence of the 2X35S-AMV promoter, which is replaced by the following double-standed BMCS oligonucleotide:

AGCTTCCCGGGGGAGCTCTAGACTCGAGCAGCT

AGGCCCTCGAGATCTGAGCTCG (SEQ ID NO:6).

Page 23 (Abstract), after the last line, beginning on a new page replace the original Sequence Listing with the substitute Sequence Listing appended herewith.

10/20/2010 10:10:10

WO 99/64451

PLANT PROTEIN WITH REPEATED WD40 MOTIFS, NUCLEIC ACID
CODING FOR SAID PROTEIN, AND USES THEREOF

5 The invention relates to the cloning of genes involved in regulating cell division in plants, and their uses.

Most plant organs develop after germination, through differentiation from the meristems. Prior to differentiation, the cell division cycle slows down and then stops in the meristems. Simultaneously, an increase in the size of the cells, and replication of the genome not accompanied by mytosis, called "endoreplication", are frequently observed. Endoreplication is a well known phenomenon during the development of storage tissue; KOWLES [Genome, 35, pp. 68-77, (1992)] thus mention a ploidy of 6C to 384C during the development of the endosperm in maize.

The phenomena involved in the stoppage of cell division preceding differentiation play an essential role in plant development and ontogeny. The mechanisms involved in these phenomena are still poorly known; it appears that the inhibition of the factor for promoting the M phase, and the induction of the protein kinases of the S phase (GRAFI, Science, 269, pp. 1262-1264, (1995)) could be involved. However, no factors directly involved in this mechanism have so far been identified in plants.

The inventors undertook the study of this mechanism with the aim of discovering the means of controlling and of acting thereby on plant development and ontogenesis.

They chose, as a study model, the *Rhizobium*/leguminous plant symbiotic system. In this system, the Nod factors, which are lipooligosaccharide in nature and which are produced by *Rhizobium*, constitute mitogenic signals which locally induce the formation of a new meristem, from which the cells forming the root nodules become differentiated [TRUCHET, Nature, 351, pp. 670-673, (1991); YANG, Plant Cell, 6, pp. 1415-1426, (1994); SAVOURE, EMBO.J., 13,

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pp. 1093-1102, (1994)]. The nodules comprise 3 main regions: an apical region, consisting of meristematic cells; an intermediate region for invasion or for differentiation (region II), where the infection of the cells by bacteria, as well as the stoppage of cell division, accompanied by endoreplication and an increase in the size of the cells, followed by their differentiation, take place; and a region for fixation (region III), consisting of differentiated cells infected by bacteria, and where the fixation of nitrogen takes place.

During this study, the inventors isolated, from lucerne (*Medicago sativa*) nodules, a gene, called hereinafter *ccs52*, which plays an essential role in the stoppage of the cell cycle and the induction of endoreplication. Using a cDNA probe of the *Medicago sativa ccs52* gene, they also isolated a homologous gene in *Medicago truncatula*.

The *ccs52* genes of *Medicago sativa* (*ccs52Ms*), and of *Medicago truncatula* (*ccs52Mt*) encode a polypeptide of 475 amino acids having a theoretical molecular mass of 52 kDa. These polypeptides are called hereinafter CCS52Ms and CCS52Mt, respectively; the sequences of CCS52Ms and CCS52Mt differ by only 2 residues at positions 16 (R/G) and 141 (V/I).

These 2 proteins comprise repeated WD motifs, and may thus be attached to the superfamily of proteins with repeated WD motifs.

The repeated WD motifs comprise about 40 amino acids containing a number of conserved amino acids including the WD motif (Trp-Asp) which is frequently situated at one end of the repeated motif [NEER et al., Nature, 371, pp. 297-300, (1994)]. The members of this family regulate various functions, such as signal transduction, transcription, pre-mRNA splicing, organization of the cytoskeleton, vesicular fusion or the cell cycle. Although the general structure is overall similar in all the proteins, the wide functional variety of repeated WD motifs suggests that

these motifs have become differentiated and have become functionally specialized. A functional homology is reflected in the number of repeated WD motifs, by a strong homology of the repeated WD motifs with
5 equivalent positions in various proteins, compared with other repeated motifs in the same proteins, and by a significant similarity of the C- and N- terminal ends.

Comparison of the sequence of CCS52Ms with the sequences of known proteins, using the GENETICS
10 COMPUTER GROUP GAP programme [parameters: gap weight: 1000; length weight: 0.100; average match: 0.540; average mismatch: 0.396] reveals a high homology with the proteins containing repeated WD40 motifs which are involved in the regulation of the cell cycle, and more
15 specifically, with the *Drosophila* FZR proteins (57% identity), *Saccharomyces cerevisiae* HCT1 (46% identity), and *Schizosaccharomyces pombe* SRW1 (52% identity), which belong to the "fizzy-related" (FZR) family. Research carried out on databases of sequences
20 using the BLAST programme [ALTSCHUL et al. Nucleic Acids Res. 25:3389-3402, (1997)] have also shown a strong homology of CCS52Ms with the *Drosophila* FZR proteins (56% identity; 70% similarity), and the *Schizosaccharomyces pombe* SRW1 proteins (51% identity;
25 67% similarity) mentioned above, as well as with the product of the *X. laevis* *fzr* gene (58% identity; 73% similarity).

The FZR proteins induce the degradation of the mitotic cyclins and are involved in the transition
30 between cell proliferation and differentiation. It has thus been shown in *Drosophila* that the *fzr* gene is expressed at the end of cell proliferation during embryogenesis. The product of this gene causes a reduction in the mitotic cyclins, and is necessary for
35 the stoppage of cell proliferation and the start of the endocycles [SIGRIST and LEHNER, Cell, 90, pp. 671-681, (1997)]. In *Saccharomyces cerevisiae*, HCT1 is necessary for the proteolysis of the mitotic cyclin, Clb2 [SCHWAB et al., Cell, 90, pp. 683-693, (1997)]. In

Schizosaccharomyces pombe, the product of the *swr1* gene controls the cell cycle and differentiation by negatively regulating the Cdc2/CDC13 (cyclin of the mitotic type) complexes [YAMAGUCHI et al., Mol. Biol. Cell., 8, 2475-2486, (1997)]. The FZR proteins therefore have a different role from that of the other proteins with repeated WD motifs, which are involved in cell proliferation.

In plants, no protein of the FZR family had been described prior to CCS52Ms.

The existence of a gene encoding a protein with repeated WD40 motifs and its isolation from carrot cDNA have recently been described [LUO et al., Plant Mol. Biol., 34, pp. 325-330, (1997)]. However, the product of this gene exhibits a weaker homology (44% identity and 63% similarity on the sequence comparison carried out with the BLAST programme) with the CCS52Ms protein than the FZR proteins of invertebrates and of yeast; this carrot protein is related to the cdc20, p55 and fizzy proteins, and therefore belongs to a subgroup of proteins with repeated WD40 motifs distinct from the FZR subgroup.

The search for homologues of CCS52Ms in a database of the *Arabidopsis thaliana* genome has revealed a peptide sequence deduced from a genomic clone (AB005230) and exhibiting 64% identity with CCS52Ms, which shows the existence of homologues of the *ccs52Ms* gene in other plants. Another peptide sequence also deduced from a genomic clone of *Arabidopsis thaliana* (AL031018, published on 17 September 1998) exhibits 80% identity with CCS52Ms (44% identity and 63% similarity based on the sequence comparison carried out with the BLAST programme).

Figure 1A represents a dendrogram of the family of proteins with repeated WD40 motifs, which shows that the CCS52 proteins form with the other FZR proteins a subfamily representing a branch which evolved separately from those respectively consisting of the CDC20, P55 and fizzy proteins.

Figures 1B and 1C represent the alignment, carried out using the "PRETTYBOX" software, of the *Medicago sativa* CCS52 (MsCCS52) sequence and the *Drosophila* FZY and FZR (DmFZY and DmFZR) sequences, of the *Saccharomyces cerevisiae* HCT1 (ScHCT1) sequence, the *Schizosaccharomyces pombe* SRW1 (SpSRW1) sequence, the *Arabidopsis thaliana* FZY (AtFZY) sequence and the 2 *Arabidopsis thaliana* polypeptides (AtCCS52A = peptide deduced from AL031018, and AtCCS52B = peptide deduced from AB005230).

The CCS52Ms protein contains 7 domains with repeated WD40 motifs, situated in the central and C-terminal portions of the molecule (the location of these domains numbered from I to VII, is indicated in Figures 1B and 1C, above the alignment of the sequences). These domains exhibit only a slight homology with each other, hence it can be concluded that they represent sites for interaction with different proteins. The latter domain (VII) comprises a potential binding site for the cyclins.

In the N-terminal portion of the CCS52Ms protein are localized a peptide sequence (DRFIPSR) which corresponds to a motif present in the FZR proteins as well as in other proteins with repeated WD40 motifs such as cdc20, p55 and fizzy, as well as a peptide sequence (AYTTLLRTALFG) which corresponds to a motif specific to the FZR family, absent from the other proteins with repeated WD40 motifs (the location of these motifs, called I and II respectively, is indicated in Figure 1B above the alignment of the sequences).

Potential sites for phosphorylation with CDKs (cyclin-dependent kinases) are located in the N-terminal portion, at positions 43 (SPSR), 99 (TPEK), 144 (SPVK), 154 (RSP) and 155 (SPYK), as well as in the C-terminal portion at position 454 (SPK), of CCS52Ms. The sites situated at positions 43 and 144 are also present in other FZR proteins, whereas the sites situated at positions 99, 154 and 155 appear more

specific to the CCS52 proteins of plants; the C-terminal site at position 454 also appears to be specific to the CCS52 proteins of plants.

5 A sequence of 15 amino acids RDNSPPPEPSPESLR starting at residue 16, and corresponding to a protein degradation motif PEST is also present in the N-terminal portion of CCS52Ms. This motif probably makes it possible, through the degradation of CCS52, to regulate its interactions with other proteins.

10 The structure of the CCS52Ms protein is schematically represented in Figure 2, in which the position of the WD40 motifs, of the phosphorylation sites (P), of the PEST motif, and of the I and II motifs, are indicated.

15 The sequence of the *Medicago sativa* cDNA cloned by the inventors is represented in the sequence listing in the annexe under the number SEQ ID NO:1; the sequence of the corresponding CCS52Ms protein is represented under the number SEQ ID NO:2.

20 The untranslated 3' region of the transcript of this DNA comprises 2 AUUUA sequences, which correspond to sequences for instability of the mRNA, and may therefore play a role in regulating the quantity of transcripts of *ccs52*.

25 The inventors searched for the presence of homologues of *ccs52Ms* by Southern transfer, in diploid and tetraploid species of *Medicago*, as well as in other plants, in particular tobacco, tomato, potato, soya, wheat and rice: in all cases, several bands were
30 detected, which indicates that *ccs52* indeed represents a family of plant genes which is related to the *fzr* family.

The inventors studied *in vivo* the activity of the CCS52Ms protein and showed that it was involved in
35 regulating cell differentiation, and in promoting endoreplication. In particular, the expression of the CCS52Ms protein in transgenic plants induces therein an increase in endoreplication and in the level of ploidy of the cells of plants. This effect could be the

consequence of a blocking of mitosis by the activation of the degradation of the mitotic cyclins, which would bring about conversion of the mitotic cycles to endocycles consisting of the G1-S-G2 phases. The result of the repetition of the endocycles is the amplification of the genome and the increase in ploidy, correlated with an increase in cell volume.

The subject of the present invention is a plant protein with repeated WD40 motifs, called CCS52, characterized in that it belongs to the FZR subfamily.

According to a preferred embodiment of the present invention, the said plant protein exhibits at least 45%, and preferably at least 55% identity with the polypeptide having the sequence SEQ ID NO:2 or at least 60% and preferably at least 70% similarity with the polypeptide having the sequence SEQ ID NO:2.

The present invention includes in particular the CCS52Ms protein, its isoforms, as well as the autologous proteins of *Medicago* and the orthologous proteins of other plants, which may be attached to the family of FZR proteins.

The invention also includes proteins derived from the CCS52 proteins by addition, deletion or substitution of one or more amino acids or of one or more amino acid sequences; this may include for example proteins in which modifications have been made outside the functional regions, or alternatively proteins in which modifications have been made in order to modify their activity, for example proteins stabilized by deletion of the PEST motif.

The subject of the present invention is also a purified nucleic acid fragment, characterized in that it comprises all or part of a sequence encoding a CCS52 protein, as defined above, or its complementary sequence. In this context, the present invention includes in particular the cDNAs and the genomic DNAs of the CCS52 proteins.

Nucleic acid fragments in accordance with the present invention can be easily identified and cloned

by screening plant cDNA or genomic DNA libraries with the aid of oligonucleotides derived from the *ccs52Ms* sequence, and in particular oligonucleotides derived from the regions of this sequence which are specific to the FZR proteins, and in particular the CCS52 proteins.

The CCS52 proteins may be produced, in particular, by expressing these nucleic acid sequences in host cells.

The subject of the present invention is also the use of a CCS52 protein, as defined above, or of a nucleic acid sequence encoding all or part of the said protein, or of its complementary sequence, for regulating the differentiation and the proliferation of plant cells.

The subject of the present invention is also the use of a protein of the FZR subfamily or of a nucleic acid sequence encoding all or part of the said protein, or of its complementary sequence, for regulating the differentiation and the proliferation of plant cells.

There may be mentioned, among such proteins, the drosophila FZR protein or the yeast FZR protein.

The modification of the expression and/or of the activity of CCS52 proteins in plant cells makes it possible to modify the cell cycle, by promoting either proliferation or differentiation, and to thus control the development process, in order to obtain, for example, stimulation of somatic embryogenesis, to increase *in vitro* regeneration of plants from calli, by increasing conversion to embryos, or to promote the development of certain organs, for example to increase the productivity of storage tissues by increasing their endoploidy.

It is possible in particular to use the cDNA sequences of CCS52 proteins or of portions of these cDNA sequences, or of their sense or antisense transcripts; this may be for example the entire sequence encoding a CCS52Ms protein, or a portion of this coding sequence, and/or all or part of the

untranslated 5' and 3' regions. These sequences may be used in the sense orientation, or if it is desired to inhibit the expression of the CCS52Ms protein in a plant or in a tissue or organ thereof, in antisense orientation.

The present invention also includes recombinant DNA constructs containing at least one nucleic acid sequence in accordance with the invention.

Generally, the said nucleic acid sequence will be placed under transcriptional control of an appropriate promoter.

Advantageously, it will thus be possible to use a strong promoter in order to increase, in the host cells, the levels of expression of the CCS52 protein; this may include an inducible promoter or a constitutive promoter, a ubiquitous promoter, or a tissue-specific promoter.

The use of inducible promoters makes it possible to obtain blocking of mitosis, and the induction of endoreplication at the desired moment. The use of tissue-specific promoters makes it possible to target the action of the CCS52 protein at certain tissues and organs (for example storage tissues).

By way of examples of strong promoters which can be used in the context of the present invention, there may be mentioned: the CaMV35S [BENFLY et al., Science, 250, pp. 959-966, (1990)], the 35S promoter; the *Agrobacterium tumefaciens* T-DNA promoters: nopaline synthase, octopine synthase, mannopine synthase, 1', 2' [SANDERS et al., Nucleic Acid Res., 15, pp. 1543-1558, (1987); HOOYKAAS and SCHILPEROORT, Plant. Mol. Biol., 19, pp. 15-38, (1992)].

By way of examples of inducible promoters which can be used in the context of the present invention, there may be mentioned: the promoter inducible by tetracycline [WEINMANN et al., Plant J., 5, pp. 559-569, (1994)]; the promoter inducible by copper [METT et al., Transgenic Res., 5, pp. 105-113, (1996)]; the

promoter inducible by glucocorticoids [AOYAMA and CHUA, Plant. J., 11, pp. 605-612, (1997)].

By way of examples of tissue-specific promoters which can be used in the context of the present invention, there may be mentioned: the endosperm-specific promoter [OPSAHL-FERSTAD et al., Plant J., 12, pp. 235-246, (1997); DOAN et al., Plant Mol. Biol., 31, pp. 877-886, (1996); the nodule-specific promoters (*enod12A/B* or *leghaemoglobin*) [TRINH et al., Plant Cell Reports, (17, pp. 345-355, (1998); VIJN et al., Plant Mol. Biol., 28, pp. 1103-1110, (1995)] or early promoters inducible by the Nod factor and late promoters (promoter of cyclin D or of late nodulins (*leghaemoglobin* type) and promoters regulated by hormones, such as *parA/B* [TAKAHASHI et al., Proc. Natl. Acad. Sci, USA, 87, pp. 8013-8016, (1990)], *GH3* [LIU et al., Plant Cell, 6, pp. 645-657, (1994)].

The invention includes in particular recombinant vectors carrying at least one insert containing a DNA fragment in accordance with the invention. These vectors can be used for transforming host cells.

The subject of the invention is also cells and pluricellular organisms transformed with at least one DNA sequence in accordance with the invention; this includes in particular plant cells or plants.

The present invention will be understood more clearly with the aid of the additional description which follows, and which refers to nonlimiting examples illustrating the identification, cloning and expression of the CCS52Ms gene.

EXAMPLE 1: CLONING AND SEQUENCING OF A CCS52MS cDNA

A cDNA clone of CCS52Ms was obtained by differential screening from a cDNA library of *Medicago sativa* nodules, highly stimulated during nodular organogenesis.

The following protocol was used:

The cDNA of *M. sativa* *ccs52Ms* was isolated by the DD-RT-PCR (Differential Display RT-PCR) technique

[LIANG and PARDEE, Science, 257, pp. 967-971, (1992)],
using the RNAimage[®] kits (GENHUNTER CORPORATION). The
RNA samples are isolated from the root region sensitive
to the Nod factor of young *M. sativa* plants (growth in
5 a nitrate-limited medium), in the absence of bacteria
or inoculated with Nod⁺ (EK1433) or Nod⁻ (EK133) strains
of *R. meliloti* for 4 days. The DD-RT-PCR *ccs52Ms*
fragment, exhibiting an increase in the expression of
the nodules, is cloned into the cloning vector pCT-TRAP
10 (GENHUNTER CORPORATION) and used as a probe for the
isolation of complete clones from a cDNA library of
nodules of *M. sativa* sp. *varia* A2, constructed in λ -ZAP
(STRATAGENE) (CRESPI et al., EMBO J., 1994, 13, 5099-
5112).

15 Seven cDNA clones, obtained from 2.10^5 phages,
represent 2 types of cDNA differing from each other
only in the 4 amino acids (16R-G, 17D-N, 33S-N, 52R-G)
and the length of the 3'UTR fragment. A 99% identity
for the clones, at the level of the amino acid
20 sequence, suggests that they represent alleles of the
same gene in allogamous tetraploid *M. sativa*.

The sequencing of the *ccs52Ms* cDNA is carried
out with the PERKIN-ELMER ABIprism system.

The genomic clones *ccs52Ms* and *ccs52Mt* are
25 isolated from genomic libraries of *M. sativa* cv.
Nagyszénasi and *M. truncatula* ecotype GHOR, using the
ccs52Ms cDNA as hybridization probe. These genomic
libraries are constructed by partial digestion of the
genomic DNA with the restriction enzyme MboI and the
30 cloning of the DNA fragments having a size of between
15 and 20 Kb into the BamHI site of λ -EMBL4.

EXAMPLE 2: IDENTIFICATION OF THE FAMILY OF THE *CCS52MS* GENE IN *MEDICAGO* AND ITS EXPRESSION IN VARIOUS PLANT ORGANS

35 The existence of multiple copies of the *ccs52*
gene is tested for by hybridization of the Southern
type in tetraploid cultivars of *M. sativa* Nagyszénasi
and Cardinal and in autogamous diploid *M. truncatula*, a
model plant in research on vegetables.

The plant DNA is isolated from young leaves, using the NUCLEON PHYTOPURE DNA extraction kit (AMERSHAM).

5 The DNA samples are digested with EcoRI and transferred onto BIOTRANS nylon membrane (+) (ICN).

The Southern hybridization is carried out in accordance with conventional protocols [(SAMBROOK, Molecular Cloning: A Laboratory Manual 2nd edn., Cold Spring Harbor Laboratory Press, New York, (1989); 10 AUSUBEL, Current Protocols in Molecular Biology, (1989)], under stringent conditions at 65°C (hybridization in CG buffer; washing: 2 x SSC, 0.1% SDS for twice 15 min, then 0.5 x SSC, 0.1% SDS for twice 30 min).

15 The expression of *ccs52Ms* is studied by Northern analysis.

Total RNA is isolated from various organs of *M. sativa* cultivar Sitel:

- from the roots, inoculated for 4 days with 20 the *R. meliloti* Nod⁻ mutant (EK133) and with the strain overproducing Nod factors (EK1433);

- from the nodules, 12, 19, 23 and 30 days after infection with *R. meliloti*, and

- from the stems, hypocotyls, leaves, buds, 25 flowers, roots of plants which are 3 days old, 7 days old, roots deprived of nitrogen and which do not have root tips, roots which are 7 days old, without root tips, placed in culture in the presence of nitrate, spontaneous nodules developed in the absence of *R. meliloti*, and root tips or a culture of cells of *M. sativa* sp. *varia* A2. 30

100 mg of each of the organs tested, collected under liquid nitrogen, are used for the extraction of the RNA (RNEASY PLANT, QUIAGEN).

35 The RNA is loaded (10 µg per lane) onto a denaturing gel (formaldehyde) [SAMBROOK, Molecular Cloning: A Laboratory Manual 2nd edn., Cold Spring Harbor Laboratory Press, New York, (1989)].

The DNA is transferred into a 10 x SSC transfer solution [CHOMCZYNSKI et al., Analytical Biochemistry, 221, pp. 303-305, (1994)].

Both in the case of the Southern hybridization and in the case of the Northern hybridization, the *ccs52Ms* cDNA fragment is labelled with [α^{32} P]dCTP (kit MEGAPRIM, AMERSHAM). Hybridization with the *Msc27* probe serves as control for the loading of the RNA [SAVOURE et al., EMBO J., 13, pp. 1093-1102, (1994)].

The results of the Southern transfer show that the probe hybridizes with various *EcoRI* fragments of the genomic DNA of *M. sativa* or *M. truncatula*, which indicates that *ccs52Ms* represents, in *Medicago*, a multigene family.

The results of the Northern transfer obtained with the total RNA of roots inoculated with the Nod⁻ EK133 mutant of *R. meliloti*, or with the EK1433 strain overproducing Nod factors and with the RNA extracted from the nodules, 12, 19, 23 and 30 days after infection with *R. meliloti* show that only a small quantity of transcripts is observed in the total RNA of the roots, which reflects the small proportion of cells involved in the organogenesis of the nodules compared with the total number of cells of the roots. By contrast, in the nodules of different ages, a high level of transcription is observed, which reflects the persistence of the apical meristems and of the regions for differentiation.

The results of the Northern transfer which are obtained with the total RNAs of: 1: culture of cells of *M. sativa* sp. *varia* A2, 2: stems, 3: hypocotyls, 4: leaves, 5: flower buds, 6: flowers, 7: roots of shoots which are 3 days old, 8: roots of shoots which are 7 days old, deprived of nitrogen, lacking ends, 9: root tips which are 7 days old, cultured in the presence of nitrates, lacking ends, 10: spontaneous nodules developed in the absence of *R. meliloti*, 11: nitrogen-fixing nodules, 12: ends of root tips, show that the expression of *ccs52Ms* is not limited to the nodules,

although this organ is that which contains the highest level of transcripts.

These transcripts are indeed present in variable quantities practically in all the organs, which indicates that this protein is involved in the development of each of them. Apart from the nodules, the level of transcription is also high in young shoots, and, in cell cultures, where a smaller sized mRNA is in addition detected which may correspond either to a different polyadenylation, or to the expression of a homologous copy of the gene.

Analyses were also carried out by *in situ* hybridization, and show that the mRNA of *ccs52Ms* is located mainly in the region for differentiation, and in particular at the interface between regions II and III of the nodule, which are regions where differentiation is the most active.

In parallel, expression of the G1 and mitotic type cyclins as well as of the H3 histone specific to the S phase is observed in the same regions.

This indicates that *CCS52Ms* is involved in the regulation of the cell cycle, probably in a manner similar to its yeast and drosophila homologues, that is to say by means of the proteolysis of mitotic cyclins, which inhibits mitosis and induces endoreplication cycles.

EXAMPLE 3: EXPRESSION OF CCS52MS IN SCHIZOSACCHAROMYCES POMBE

The expression of *CCS52Ms* was studied in *S. pombe* in which a functional homologue (*SRW1*) was recently described (YAMAGUCHI, publication cited above). The gene encoding *CCS52Ms* was cloned into the plasmid into *pREP1* under the control of the *nmf1* promoter which is repressible by thiamine.

The cDNA of *ccs52Ms* obtained after cleavage of λ -ZAP (STRATAGENE) is digested with AgeI and partially with EcoRV. The AgeI-EcoRV fragment of 1.6 kb representing the coding region, with the exception of the first 4 codons, is cloned into a vector SKII

BLUESCRIPT (STRATAGENE) digested with XmaI (compatible
with AgeI) and EcoRV. From this plasmid (pSK52B), the
cDNA of *ccs52Ms* is cut by BamHI-EcoRV digestion and
cloned into the BamHI-SmaI sites of the plasmid pREP1
5 [MAUNDRELL et al., Gene, 123, pp. 127-30, (1993)]. To
generate an open reading frame in phase with the ATG
codon for translation present in the vector under the
control of the *nmtI* promoter, the DNA is digested with
BamHI and the 5' end is completed in the presence of
10 the Klenow enzyme and of dNTPs. The religation of the
blunt ends causes correct fusion, also verified by
sequencing. This plasmid, called pREP52, is used to
transform competent *S. pombe* SP-Q01 cells and the
transformants are selected on EMM-thiamine agar plates,
15 using the ESP kit (STRATAGENE). The vectors pREP1
[MAUNDRELL et al., Gene, 123, pp. 127-30, (1993)] and
pESP1 (STRATAGENE) are used as negative controls; the
positive control consists of *srw1* cloned into pREP1
[YAMAGUSHI et al., Mol. Biol. Cell., 8, pp. 2475-2486,
20 (1997)].

The transformants of *S. pombe* SP-Q01 are
cultured in 2 ml of 5 μ M EMM-thiamine medium for 32 h
at 30°C. The cells are washed twice with 10 ml of
sterile water and resuspended in 5 ml of EMM medium.
25 The cellular suspensions are divided into two halves:
2.5 ml are cultured with thiamine and 2.5 ml are
cultured without thiamine, at 30°C. Culture aliquots
are collected after 16 h and 24 h of culture and fixed
with ethanol, stained with DAPI or with propidium
30 iodide for analysis by flow cytometry and by microscopy
[BEACH et al., Curr. Genet., 10, pp. 297-311, 1985)].

In the presence of thiamine, the expression of
CCS52Ms is repressed and normal growth is observed.

In the absence of thiamine, the expression of
35 CCS52Ms causes the inhibition of the growth of
S. pombe, which is accompanied by endoreplication as
illustrated in Figure 3B, which shows the presence of
nuclei $\geq 4C$, which is not observed in the control cells

of *S. pombe*, carrying the empty vector pREP1 (Figure 3A).

The morphology of the cells is also modified by the expression of CCS52Ms. A lengthening of the cells and an increase in the size of the nuclei are observed, which are identical to those observed during the expression of SRW1 [YAMAGUSHI et al., Mol. Biol. Cell., 8, pp. 2475-2486, (1997)], whereas no morphological change is observed when *S. pombe* carries only the vector pREP1.

In *S. pombe*, SRW1 is essential for the degradation of the mitotic cyclin CDC13. To verify if CCS52 acts in the same manner, the quantity of the CDC13 was evaluated in cultures of a strain (SY1) of *S. pombe*, carrying a deletion in the *srw1* gene, and not degrading CDC13.

The total proteins obtained from cultures of SY1 transformed with pREP1 (control) or with pREP1-ccs52 was analysed by Western transfer, and visualized with the aid of anti-CDC13 antibodies.

In parallel, the expression of CDC2 kinase and that of α -tubulin were evaluated by visualization with the aid of anti-PSTAIR and anti- α -tubulin antibodies, respectively.

The results obtained show a very high reduction in CDC13 in the cells transformed with pREP1-ccs52 compared with the control cells. By contrast, there is no variation in CDC2 and in α -tubulin.

These results confirm that CCS52 is a functional equivalent of SRW1.

EXAMPLE 4: PRODUCTION OF TRANSGENIC PLANTS TRANSFORMED WITH THE CCS52MS GENE

1. Expression of an antisense transcript and its action on the level of ploidy of *Medicago truncatula*.

In a first instance, the level of ploidy of various organs of *Medicago truncatula* (plant which is naturally diploid) was determined, by flow cytometry, in nontransformed plants.

The technique used is the following:

The nuclear DNA of freshly harvested plants is analysed by flow cytometry (EPICS V, Coulter), in accordance with the method of BROWN et al., (A laboratory guide for Cellular and Molecular plant Biology, 1991, 326-345, ed. Negrutiu et al., Birkhäuser, Basel), modified such that the nuclei are stained with DAPI at a final concentration of 5 µg/ml. The nuclear buffer I is used at 1% Triton X-100 for the nodules.

10 In young shoots, a quantity of DNA from 2C to 8C is found in the root and the cotyledon, whereas the hypocotyl also contains nuclei at 16C. In adult plants, the leaves are diploid, containing 95% of nuclei at 2C and 5% of nuclei at 4C. In the petioles and the nodules, nuclei from 2C to 32C were detected. However, 15 the petiole contains predominantly nuclei at 2C, whereas the nodules contain predominantly nuclei at 4C.

An SstI-PvuII fragment of 1.2 kb containing 3/4 of the coding sequence of *ccs52Ms*, was placed in 20 antisense orientation under the control of the 35S promoter, in a binary vector obtained from the vector pGPTV-BAR, carrying the *bar* gene for resistance to the herbicide BASTA as selectable marker, and multiple cloning sites. This construct is obtained by inserting 25 the 35S promoter into a HindIII-XbaI fragment (obtained from pBI121, CLONTECH), into the HindIII-XbaI sites of the vector pGPTV-BAR. The *uidA* gene is then removed from the plasmid pGPTV-BAR by XbaI-SstI digestion at the level of the multiple cloning site.

30 To obtain the antisense construct of *ccs52Ms*, the SstI-PvuII fragment of 1.2 kb is cloned into the SmaI-SstI sites of the binary vector thus obtained.

These plasmids as well as a control plasmid, containing the *gus* gene instead of the antisense *ccs52* 35 construct were introduced into *Agrobacterium tumefaciens* (EHA105) by electroporation and used to transform *Medicago truncatula* R108-1 according to the protocol described by HOFFMANN et al. [Mol. Plant

Microbe Interaction, 10, pp. 307-315, (1997)]; TRINH ET AL. [Plant Cell Reports, 17, pp. 345-355, (1998)].

The level of ploidy of the transgenic plants obtained was analysed, as described above and the level of endogenous transcripts was evaluated by RT-PCR. To differentiate the endogenous transcripts of *ccs52Mt* from the antisense transcripts, the pair of primers P55CL/P55CR is used for the endogenous transcripts and the pair of primers P55BL/P55CR for the antisense transcripts.

P55BL : TTTGGGGGTTGATGATTGTG
P55CL : CTCTCTACCGTTCTATCTCTTGGGA
P55CR : GGTAAGATGCTACTTTTGGTGGTGT

The position of these primers is schematically represented in Figure 4.

Figure 5A shows the results of evaluation of the quantity of endogenous *ccs52Mt* transcripts:

- by RT-PCR (□) in the transgenic lines A1, A3, A4, A7 and A32 and in the control plants containing the *gus* gene (C_{2n}), and
- by Northern transfer (■) in A4 and C_{2n} plants.

The results of analysis by flow cytometry are illustrated by Figure 5B, for the petioles of control plants containing the *gus* gene, diploids (C_{2n}) or tetraploids (C_{4n}), and of plants of the A4 line.

Out of 38 regenerated transgenic plants, 3 (A4, A7 and A32) showed a significantly reduced endoploidy, and in particular the plant A4. It is also in this line that the level of expression of the endogenous transcripts of *ccs52Ms* is the lowest, as shown in Figure 5B. The fact that a reduction in endoploidy was never observed before in other transgenic plants and are not observed in the control plants makes it possible to attribute this phenomenon to the impairment of the expression of *CCS52Ms*, and not to a secondary effect of transgenesis.

In addition, the plant A4 produces a quantity of seeds significantly less than that of the control plants. Moreover, it forms fewer side branches, and

develops only 2 nodules at the level of the roots, instead of the 50 nodules on average developed by the control plants cultured under the same conditions.

The impact of the partial suppression of the expression of *ccs52* on the development of the plant organs was also determined. For this purpose, the width of the petioles was measured and correlated with the percentages of endoreplicated nuclei ($> 4C$), in the T1 generation derived from the A4 line and in the C_{2n} and C_{4n} control plants.

The results are illustrated in Figure 6. Figure 6A which represents the width of the petiole as a function of the percentage of polyploid cells shows that, in the C_{2n} control plants (18 plants), the width of the petioles varies in correlation with the number of diploid cells. In the plants derived from A4 (36 plants), a more reduced variation in the size of the petioles and a lower percentage of polyploid cells are observed, which indicates that the degree of endoploidy can directly affect the final size of the plant organs.

12 of the 36 T1 plants derived from A4 contain less than 6% of endoreplicated nuclei ($> 4C$) in their petioles (Figure 6B). These plants [A4(s)] were grouped together and analysed separately from the rest of the A4 T1 plants [A4(w)] which exhibit less substantial phenotypic impairments.

Figure 6C shows that the width of the petioles in A4(w) plants is comparable to that of the diploid C_{2n} control plants; by contrast, the width of the petioles in the A4(s) plants is significantly less than that of the diploid C_{2n} control plants and the width of the petioles in the tetraploid C_{4n} control plants is significantly greater than that observed in the diploid plants.

The size of the leaves (which do not contain endoreplicated cells and whose endoploidy is not therefore affected by the level of expression of *CCS52*) was also measured. In this case, no significant difference is observed between the A4(w) plants, the

A4(s) plants and the diploid C_{2n} control plants; by contrast, the size of the leaves is significantly larger in the tetraploid C_{4n} control plants.

These results show that endoploidy affects the size of the plant organs, and that the modification of the expression of CCS52 acts at this level through a modification of the endoploidy.

2. Expression of the CCS52Ms protein in transgenic plants.

Expression vectors containing the *ccs5Ms* gene under the control of the 35S promoter, as well as expression vectors containing the *ccs52Ms* gene, under the control of a tissue-specific promoter, were constructed according to the following protocol:

For the tissue-specific expression of CCS52Ms, the cDNA is placed under the control of the *enod12AMs* and *Srg1b3* promoters described by TRINH et al. [Plant Cell Reports, 17, pp. 345-355, (1998)], using as a vector pISV-BMCS, a derivative of pISV2301, and, instead of the complete *enod12AMs* promoter, only one 0.3 kb fragment thereof, considered to be sufficient for a nodule-specific expression [VIJN et al., Plant Mol. Biol., 28, pp. 1103-1110, (1995)].

Construction of pISV-BMCS: pISV2301 is digested with HindIII and SstI in order to eliminate the sequence of the 2X35S-AMV promoter, which is replaced by the following double-stranded BMCS oligonucleotide:

AGCTTCCCGGGGAGCTCTAGACTCGAGCAGCT
AGGCCCTCGAGATCTGAGCTCG.

This oligonucleotide contains the SmaI, SstI, XbaI and XhoI sites.

pISV-BMCS12A is constructed by cloning into pISV-BMCS of a fragment of the 0.3 kb of the *enod12AMs* promoter, obtained from the plasmid pPR89 [BAUER et al., Plant J., 10, pp. 91-105, (1996)].

pISV-BMCS-LB3 is constructed by digestion of pISV-BMCS with HindIII-SstI and cloning of a HindIII-SstI fragment containing the leghaemoglobin promoter of

Sesbania rostrata from pLP32 [TRINH et al., Plant Cell Reports, 17, pp. 345-355, (1998)].

5 These vectors were used to transform *Medicago truncatulata* according to the protocol described above for the antisense sequences.

10 During the regeneration of the transgenic plants, a significantly greater conversion of the calli to embryos is observed in plants transformed with the constructs expressing the *ccs52Ms* gene, than in plants transformed with the control construct, which indicates a positive effect of CCS52Ms on somatic embryogenesis.

CLAIMS

1. Plant protein with repeated WD40 motifs, characterized in that it belongs to the FZR subfamily.
- 5 2. Protein according to Claim 1, characterized in that it exhibits at least 45%, and preferably at least 55%, identity with the polypeptide having the sequence SEQ ID No. 2 or at least 60% and preferably at least 70% similarity with the polypeptide having the sequence
10 SEQ ID No. 2.
3. Purified nucleic acid fragment, characterized in that it comprises all or part of a sequence encoding a protein according to Claim 1, or its complementary sequence.
- 15 4. Recombinant vector containing a nucleic acid fragment according to Claim 3.
5. Cell transformed with at least one nucleic acid fragment according to Claim 3.
6. Cell transformed according to Claim 5,
20 characterized in that it is a plant cell.
7. Transgenic plant transformed with at least one nucleic acid fragment according to Claim 3.
8. Use of a protein according to either of Claims 1 and 2, or of a nucleic acid sequence according to
25 Claim 3, for regulating the differentiation and the proliferation of plant cells.
9. Use according to Claim 8, characterized in that the said protein or the said nucleic acid sequence is used to promote endoploidy in the cells of a plant or
30 of a plant tissue.
10. Use according to Claim 8, characterized in that the said protein or the said nucleic acid sequence is used to promote the *in vitro* regeneration of plants from calli in culture.
- 35 11. Use of a protein of the FZR subfamily or of a nucleic acid sequence encoding all or part of the said protein, or its complementary sequence, for regulating the differentiation and the proliferation of plant cells.

T06370-2257060

ABSTRACT

The invention concerns a plant protein with repeated WD40 motifs, characterised in that it belongs to the FZR sub-family, a purified nucleic acid fragment characterised in that it comprises all or part of a sequence coding for said plant protein and the uses of said protein and said nucleic acid fragment.

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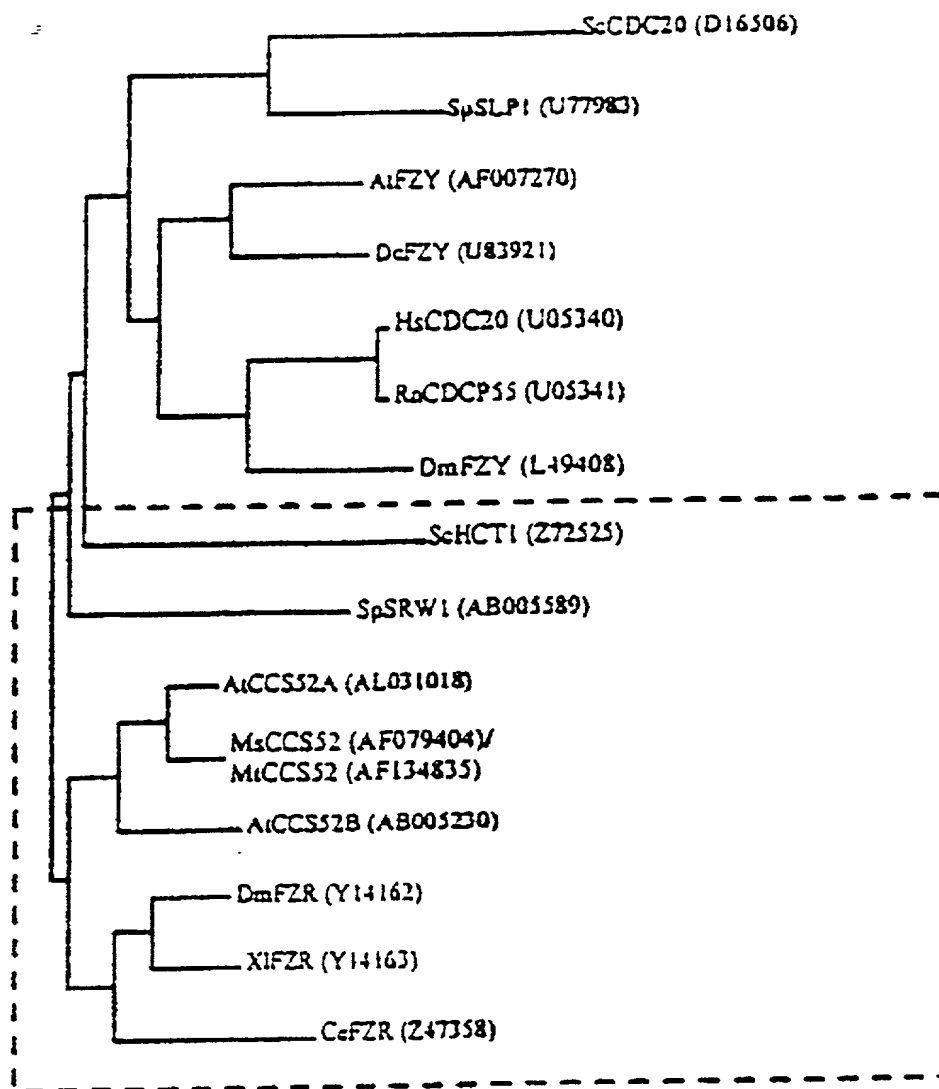


Figure 1A

09701572-012901

1	AC72Y	1	MSQPNFV8DLQNALIMDOETROPAPRMKKKLEASLNO8VNTTABVLS	VRATCTVPEZHFLEPRKKLEKROHL	...
1	DM72Y	1	...	OVQAPRTKTPOKSSZQKTKKSNTTFS	...
1	MC852	1	...	PEBLKHVSRRMINBNHYTBS	...
1	AC852A	1	...	MANLSPAMNTPV8LEBRINRLINANQSOBSP8	...
1	AC852B	1	...	MQTSLRLT78887KQIGBL	...
1	DM72R	1	...	MP8EPEYOKAILKHYBPVARNLPHNPF	...
1	SP8AM1	1	...	RODABTRMNOQF8K87P	...
1	SC8CT1	1	...	KRYABKNYIS8888ABLL	...
22	AT72Y	22	DRFIPMRS	VT888KAYMT	...
22	DM72Y	22
22	MC852	22
22	AC852A	22
22	AC852B	22
22	DM72R	22
22	SP8AM1	22
22	SC8CT1	22
62	AT72Y	62	QIAVVMHQNATRIILA
62	DM72Y	62
62	MC852	62
62	AC852A	62
62	AC852B	62
62	DM72R	62
62	SP8AM1	62
62	SC8CT1	62
97	AT72Y	97	PKPVP
97	DM72Y	97
97	MC852	97
97	AC852A	97
97	AC852B	97
97	DM72R	97
97	SP8AM1	97
97	SC8CT1	97

Figure 1B

WD40-2		
118	ATZY	DABEORTS ELVTIDEDKO WTSIMT DPCLD LAUGLDNBEV OIMDC VSNRQVRTL RGGH SRVGLANDHNI LTTGOMIQ
119	DMZY	NAQTONIA QLTAPERO DYAQSLAM IOEQI LAEGNLTGAU ELMDCA RVGLANNSV LYSBOSRIO
120	MCCB52	NAQSSKVT RLCDDOVD DQUCSVQWADRCCTHLAVGT MHGVCV OIMDC AARCKKIRHSH DGHARVGLANNSV LYSBOSRIO
121	ATCCB52A	NAQSSKVT RLCDDOVD DQUCSVQWADRCCTHLAVGT MHGVCV OIMDC AARCKKIRHSH DGHARVGLANNSV LYSBOSRIO
122	ATCCB52B	NAQSSKVT RLCDDOVD DQUCSVQWADRCCTHLAVGT MHGVCV OIMDC AARCKKIRHSH DGHARVGLANNSV LYSBOSRIO
123	DMZFA	NAQSSKVT RLCDDOVD DQUCSVQWADRCCTHLAVGT MHGVCV OIMDC AARCKKIRHSH DGHARVGLANNSV LYSBOSRIO
124	SPARM1	NAQSSKVT RLCDDOVD DQUCSVQWADRCCTHLAVGT MHGVCV OIMDC AARCKKIRHSH DGHARVGLANNSV LYSBOSRIO
125	5CHCT1	NAQSSKVT RLCDDOVD DQUCSVQWADRCCTHLAVGT MHGVCV OIMDC AARCKKIRHSH DGHARVGLANNSV LYSBOSRIO
WD40-3		
228	ATZY	KIVMNDVRI R98IURT YIGHTMEVCCGLNBSOMKQASGGNDRVVMIMDLASBKKQTQMLHREFFRENTAAVKAALAWSP
229	DMZY	TIVMNDVRI R98IURT YIGHTMEVCCGLNBSOMKQASGGNDRVVMIMDLASBKKQTQMLHREFFRENTAAVKAALAWSP
230	MCCB52	TIVMNDVRI R98IURT YIGHTMEVCCGLNBSOMKQASGGNDRVVMIMDLASBKKQTQMLHREFFRENTAAVKAALAWSP
231	ATCCB52A	TIVMNDVRI R98IURT YIGHTMEVCCGLNBSOMKQASGGNDRVVMIMDLASBKKQTQMLHREFFRENTAAVKAALAWSP
232	ATCCB52B	TIVMNDVRI R98IURT YIGHTMEVCCGLNBSOMKQASGGNDRVVMIMDLASBKKQTQMLHREFFRENTAAVKAALAWSP
233	DMZFA	TIVMNDVRI R98IURT YIGHTMEVCCGLNBSOMKQASGGNDRVVMIMDLASBKKQTQMLHREFFRENTAAVKAALAWSP
234	SPARM1	TIVMNDVRI R98IURT YIGHTMEVCCGLNBSOMKQASGGNDRVVMIMDLASBKKQTQMLHREFFRENTAAVKAALAWSP
235	5CHCT1	TIVMNDVRI R98IURT YIGHTMEVCCGLNBSOMKQASGGNDRVVMIMDLASBKKQTQMLHREFFRENTAAVKAALAWSP
WD40-4		
307	ATZY	FOASGLAAGGGVGRDKIRFNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
308	DMZY	FOASGLAAGGGVGRDKIRFNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
309	MCCB52	FOASGLAAGGGVGRDKIRFNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
310	ATCCB52A	FOASGLAAGGGVGRDKIRFNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
311	ATCCB52B	FOASGLAAGGGVGRDKIRFNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
312	DMZFA	FOASGLAAGGGVGRDKIRFNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
313	SPARM1	FOASGLAAGGGVGRDKIRFNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
314	5CHCT1	FOASGLAAGGGVGRDKIRFNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
WD40-5		
407	ATZY	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
408	DMZY	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
409	MCCB52	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
410	ATCCB52A	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
411	ATCCB52B	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
412	DMZFA	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
413	SPARM1	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
414	5CHCT1	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
WD40-6		
507	ATZY	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
508	DMZY	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
509	MCCB52	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
510	ATCCB52A	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
511	ATCCB52B	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
512	DMZFA	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
513	SPARM1	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
514	5CHCT1	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
WD40-7		
607	ATZY	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
608	DMZY	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
609	MCCB52	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
610	ATCCB52A	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
611	ATCCB52B	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
612	DMZFA	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
613	SPARM1	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER
614	5CHCT1	WOPSTLASEGGGTADRCIA FNNITAGACINBSVATGSOVCCDLNSQBSNELLSSHGP TQROL TLWRYPSHSHRMAH LHTGHTER

Figure 1C

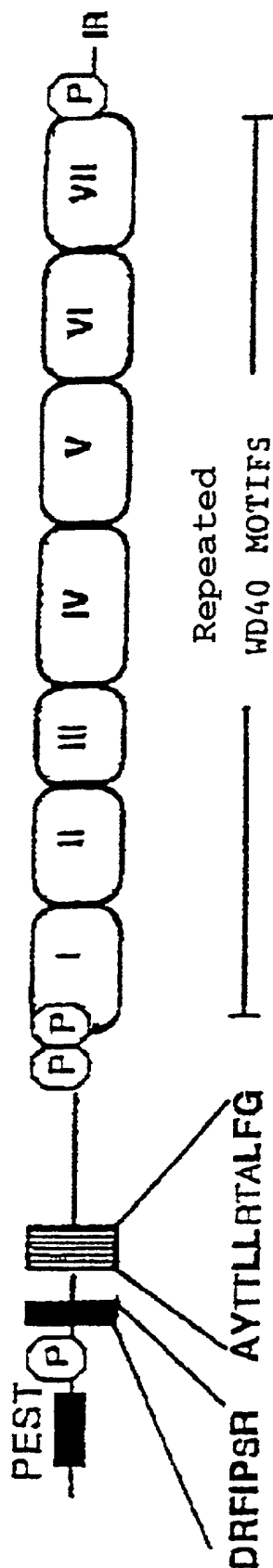


Figure 2

Figure 2

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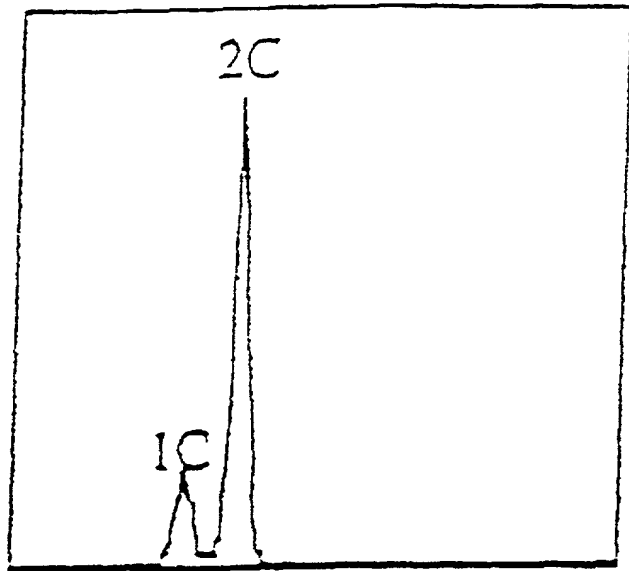


Figure 3A

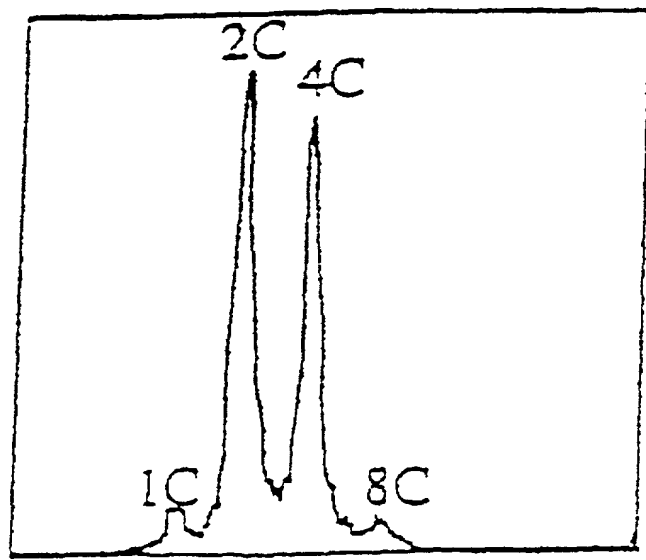


Figure 3B

09/701572-24510460

TOP SECRET 2/5/70/60

6/8

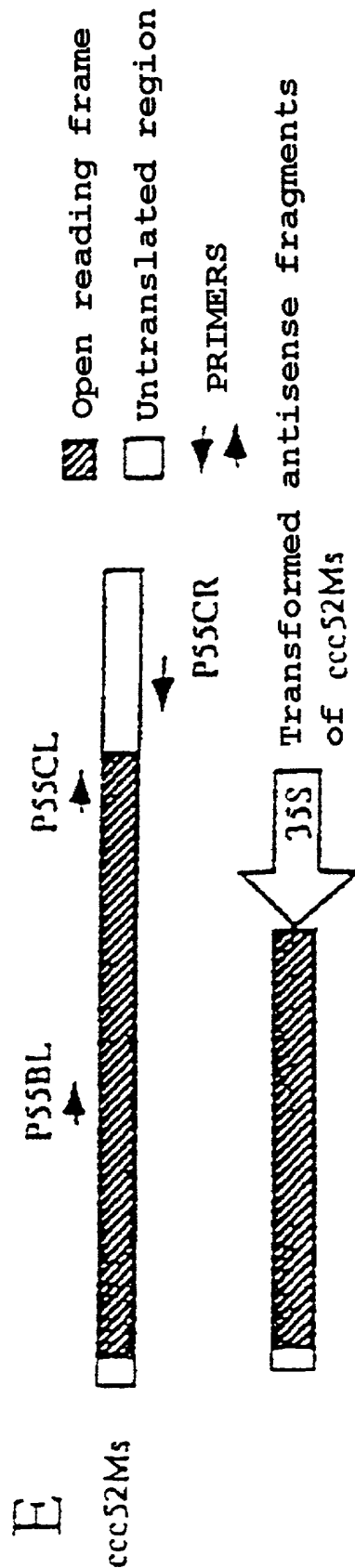
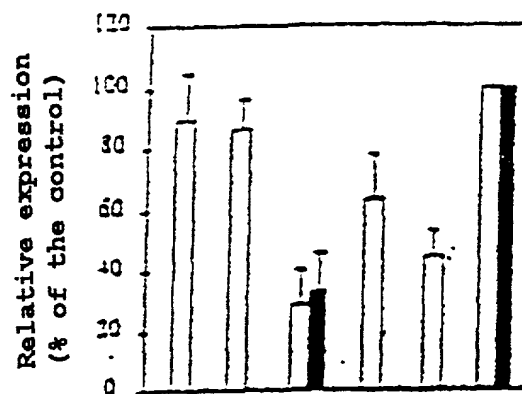


Figure 4

Figure 4



% Nuclei	A1	A3	A4	A7	A32	C ₂
8c	13.6	13.6	1.2	13.3	7.5	15.8
16c	3.8	3.2	0	0.5	0	4.1

Figure 5A

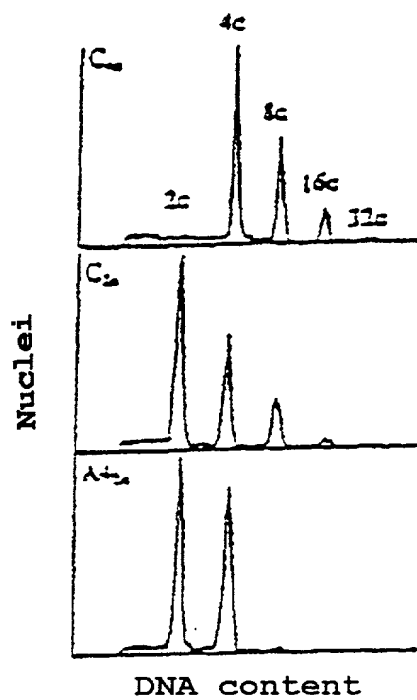


Figure 5B

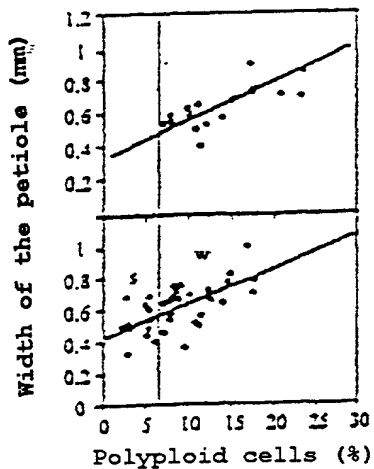


Figure 6A

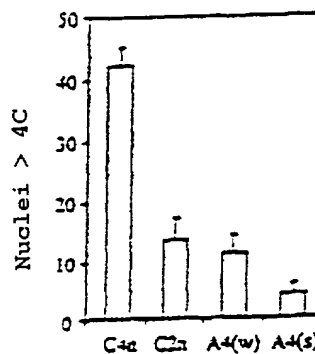


Figure 6B

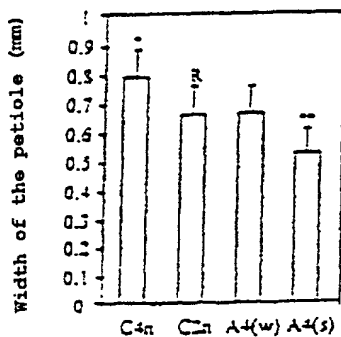


Figure 6C

FIGURE 6A

Declaration and Power of Attorney for Patent Application
Déclaration et Pouvoirs pour Demande de Brevet
French Language Declaration

En tant l'inventeur nommé ci-après, je déclare par le présent acte que :

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

et dont la description est fournie ci-joint à moins

- ☐ ci-joint
☐ a été déposée le

sous le numéro de demande des
Etats-Unis ou le numéro de demande
international PCT

et modifiée le

(le cas échéant).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait références ci-dessus.

Je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

As a below named inventor, I hereby declare that :

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed an for which a patent is sought on the invention entitled

**Plant protein with repeated WD40 motifs,
nucleic acid coding for said protein, and
uses thereof**

the specification of which :

- ☐ is attached hereto.
☒ was filed on

as United States Application Number or
PCT International Application Number.
PCT/FR99/01342 filed on **June 8, 1999**

and was amended on

(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

Prior Foreign application(s)

Demande(s) de brevet antérieure(s) dans un autre pays.

(Number) (Country)
(Numéro) (Pays)

98 07174 FRANCE

(Number) (Country)
(Numéro) (Pays)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

(Application No.) (Filing Date)
(N° de demande) (Date de dépôt)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, § 365(c) du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, § 112 du code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande :

(Application No.) (Filing Date)
(N° de demande) (Date de dépôt)

(Application No.) (Filing Date)
(N° de demande) (Date de dépôt)

Je déclare que par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique ; et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la section 1001 du Titre 18 du Code des Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Priority claimed
Droit de priorité
revendiqué

(Day/Month/Year Filed) ☒ ☐
(Jour/Mois/Anné de dépôt) Yes No
Oui Non

08.06.1998

(Day/Month/Year Filed) ☐ ☐
(Jour/Mois/Anné de dépôt) Yes No
Oui Non

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (Filing Date)
(N° de demande) (Date de dépôt)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Status) (patented, pending, abandoned)
(Statut) (breveté, en cours d'examen, abandonné)

(Status) (patented, pending, abandoned)
(Statut) (breveté, en cours d'examen, abandonné)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

French Language Declaration

POUVOIRS : En tant que l'inventeur cité, je désigne par la présente l'(les) avocat(s) et/ou agent(s) suivant(s) pour qu'ils poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire s'y rapportant avec l'Office des brevets et des marques : (mentionner le nom et le numéro d'enregistrement).

POWER OF ATTORNEY : As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to persecute this application and transact all business in the Patent and Trademark Office connected therewith : (list name and registration number)

Norman F. Oblon, Reg. No. 24,618; Marvin J. Spivak, Reg. No. 24,913; C. Irvin McClelland, Reg. No. 21,124; Gregory J. Maier, Reg. No. 25,599; Arthur I. Neustadt, Reg. No. 24,854; Richard D. Kelly, Reg. No. 27,757; James D. Hamilton, Reg. No. 28,421; Eckhard H. Kuesters, Reg. No. 28,870; Robert T. Pous, Reg. No. 29,099; Charles L. Gholz, Reg. No. 26,395; William E. Beaumont, Reg. No. 30,996; Jean-Paul Lavalleye, Reg. No. 31,451; Stephen G. Baxter, Reg. No. 34,884; Richard L. Treanor, Reg. No. 36,379; Stephen P. Wehrhouch, Reg. No. 32,829; John T. Goolkasian, Reg. No. 26,142; Richard L. Cinn, Reg. No. 34,305; Stephen E. Lipman, Reg. No. 30,011; Carl E. Shlier, Reg. No. 34,426; James J. Kubaski, Reg. No. 34,648; Richard A. Neifeld, Reg. No. 35,299; J. Dereck Mason, Reg. No. 35,270; Surinder Sachar, Reg. No. 34,423; Christina M. Gadiano, Reg. No. 37,628; Jeffrey B. McIntyre, Reg. No. 36,867; William T. Enos, Reg. No. 33,128; Michael E. McCabe, Jr., Reg. No. 37,182; Bradley D. Lytle, Reg. No. 40,073; and Michael R. Asey, Reg. No. 40,294, with full powers of substitution and revocation.

Addresser toute correspondance à :

Send Correspondence to :

OBLON, SPIVAK, MCCLELLAND, MAIER & NEUSTADT, P.C.
FOURTH FLOOR
1755 JEFFERSON DAVIS HIGHWAY
ARLINGTON, VIRGINIA 22202 U.S.A.

Adresser tout appel téléphonique à :
 (nom et numéro de téléphone)

Direct Telephone calls to : (name and telephone number)

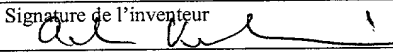
(703) 413-3000

Nom complete de l'unique ou premier inventeur Eva KONDOROSL		Full name of sole or first inventor	
Signature de l'inventeur <i>Eva Kondorosl</i>	Date <u>30/11/2000</u>	Inventor's signature	Date
Domicile F-91190 Gif sur Yvette (FRANCE) <i>FRX</i>		Residence	
Nationalité Hongroise		Citizenship	
Adresse Postale 10, allée de la Dame Alips F-91190 Gif sur Yvette (FRANCE)		Post Office Address	
Nom complete du second co-inventeur, le cas echeant Angel CEBOLLA		Full name of second joint inventor, if any	
Signature de l'inventeur	Date	Second inventor's signature	Date
Domicile F-91190 Gif sur Yvette (FRANCE)		Residence	
Nationalité Espagnole		Citizenship	
Adresse Postale 15, rue Juliette Adam F-91190 Gif sur Yvette (FRANCE)		Post Office Address	

(Fournier les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

French Language Declaration

300 Nom complete du troisième co-inventeur, le cas échéant Adam KONDOROSI		Full name of third joint inventor, if any	
Signature de l'inventeur 	Date 30/11/2000	Third inventor's signature	Date
Domicile F-91190 Gif sur Yvette (FRANCE) FRX		Residence	
Nationalité Hongrois		Citizenship	
Adresse Postale 10, allée de la Dame Alips F-91190 Gif sur Yvette (FRANCE)		Post Office Address	
Nom complete du quatrième co-inventeur, le cas échéant		Full name of fourth joint inventor, if any	
Signature de l'inventeur	Date	Fourth inventor's signature	Date
Domicile		Residence	
Nationalité		Citizenship	
Adresse Postale		Post Office Address	
Nom complete du cinquième co-inventeur, le cas échéant		Full name of fifth joint inventor, if any	
Signature de l'inventeur	Date	Fifth inventor's signature	Date
Domicile		Residence	
Nationalité		Citizenship	
Adresse Postale		Post Office Address	
Nom complete du sixième co-inventeur, le cas échéant		Full name of sixth joint inventor, if any	
Signature de l'inventeur	Date	Sixth inventor's signature	Date
Domicile		Residence	
Nationalité		Citizenship	
Adresse Postale		Post Office Address	

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

Declaration and Power of Attorney for Patent Application
Déclaration et Pouvoirs pour Demande de Brevet
French Language Declaration

En tant l'inventeur nommé ci-après, je déclare par le présent acte que :

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

et dont la description est fournie ci-joint à moins

- ☐ ci-joint
☐ a été déposée le

sous le numéro de demande des Etats-Unis ou le numéro de demande international PCT

et modifiée le

(le cas échéant).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait références ci-dessus.

Je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

As a below named inventor, I hereby declare that :

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed an for which a patent is sought on the invention entitled

Plant protein with repeated WD40 motifs, nucleic acid coding for said protein, and uses thereof

the specification of which :

- ☐ is attached hereto.
☒ was filed on

as United States Application Number or PCT International Application Number.
PCT/FR99/01342 filed on **June 8, 1999**

and was amended on

(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

Prior Foreign application(s)

Demande(s) de brevet antérieure(s) dans un autre pays.

(Number) (Country)
(Numéro) (Pays)

98 07174 FRANCE

(Number) (Country)
(Numéro) (Pays)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

(Application No.) (Filing Date)
(N° de demande) (Date de dépôt)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, § 365(c) du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, § 112 du code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande :

(Application No.) (Filing Date)
(N° de demande) (Date de dépôt)

(Application No.) (Filing Date)
(N° de demande) (Date de dépôt)

Je déclare que par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la section 1001 du Titre 18 du Code des Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Priority claimed
Droit de priorité
revendiqué

(Day/Month/Year Filed) ☒ ☐
(Jour/Mois/Année de dépôt) Oui Non

08.06.1998

(Day/Month/Year Filed) ☐ ☐
(Jour/Mois/Année de dépôt) Oui Non

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (Filing Date)
(N° de demande) (Date de dépôt)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Status) (patented, pending, abandoned)
(Statut) (breveté, en cours d'examen, abandonné)

(Status) (patented, pending, abandoned)
(Statut) (breveté, en cours d'examen, abandonné)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

French Language Declaration

POUVOIRS : En tant que l'inventeur cité, je désigne par la présente l'(les) avocat(s) et/ou agent(s) suivant(s) pour qu'ils poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire s'y rapportant avec l'Office des brevets et des marques : (mentionner le nom et le numéro d'enregistrement).

POWER OF ATTORNEY : As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to persecute this application and transact all business in the Patent and Trademark Office connected therewith : (list name and registration number)

Norman F. Oblon, Reg. No. 24,618 ; Marvin J. Spivak, Reg. No. 24,913 ; C. Irvin McClelland, Reg. No. 21,124 ; Gregory J. Maier, Reg. No. 25,599 ; Arthur I. Neustadt, Reg. No. 24,854 ; Richard D. Kelly, Reg. No. 27,757 ; James D. Hamilton, Reg. No. 28,421 ; Eckhard H. Kuesters, Reg. No. 28,870 ; Robert T. Pous, Reg. No. 29,099 ; Charles L. Gholz, Reg. No. 26,395 ; William E. Beaumont, Reg. No. 30,996 ; Jean-Paul Lavalleye, Reg. No. 31,451 ; Stephen G. Baxter, Reg. No. 34,884 ; Richard L. Treanor, Reg. No. 36,379 ; Stephen P. Weihrouh, Reg. No. 32,829 ; John T. Goolkasian, Reg. No. 26,142 ; Richard L. Cinn, Reg. No. 34,305 ; Stephen E. Lipman, Reg. No. 30,011 ; Carl E. Shlier, Reg. No. 34,426 ; James J. Kubaski, Reg. No. 34,648 ; Richard A. Neifeld, Reg. No. 35,299 ; J. Dereck Mason, Reg. No. 35,270 ; Surinder Sachar, Reg. No. 34,423 ; Christina M. Gadiano, Reg. No. 37,628 ; Jeffrey B. McIntyre, Reg. No. 36,867 ; William T. Enos, Reg. No. 33,128 ; Michael E. McCabe, Jr., Reg. No. 37,182 ; Bradley D. Lytle, Reg. No. 40,073 ; and Michael R. Asey, Reg. No. 40,294, with full powers of substitution and revocation.

Addresser toute correspondance à :


Send Correspondence to :

OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C.
FOURTH FLOOR
1755 JEFFERSON DAVIS HIGHWAY
ARLINGTON, VIRGINIA 22202 U.S.A.

Adresser tout appel téléphonique à :
(nom et numéro de téléphone)

Direct Telephone calls to : (name and telephone number)

(703) 413-3000

Nom complete de l'unique ou premier inventeur Eva KONDOROSI	Full name of sole or first inventor
Signature de l'inventeur _____ Date _____	Inventor's signature _____ Date _____
Domicile F-91190 Gif sur Yvette (FRANCE)	Residence
Nationalité Hongroise	Citizenship
Adresse Postale 10, allée de la Dame Alips F-91190 Gif sur Yvette (FRANCE)	Post Office Address
Nom complete du second co-inventeur, le cas echeant Angel CEBOLLA	Full name of second joint inventor, if any
Signature de l'inventeur  Date 27 DEC 2000	Second inventor's signature _____ Date _____
Domicile 41013 Séville (Espagne) ESX	Residence
Nationalité Espagnole	Citizenship
Adresse Postale C/ Parroco Ant. Gzlez Abato, 18. 1°C, 41013 Séville (ESAPGNE)	Post Office Address

(Fournier les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

French Language Declaration

Nom complete du troisième co-inventeur, le cas échéant Adam KONDOROSI		Full name of third joint inventor, if any	
Signature de l'inventeur	Date	Third inventor's signature	Date
Domicile F-91190 Gif sur Yvette (FRANCE)		Residence	
Nationalité Hongrois		Citizenship	
Adresse Postale 10, allée de la Dame Alips F-91190 Gif sur Yvette (FRANCE)		Post Office Address	
Nom complete du quatrième co-inventeur, le cas échéant		Full name of fourth joint inventor, if any	
Signature de l'inventeur	Date	Fourth inventor's signature	Date
Domicile		Residence	
Nationalité		Citizenship	
Adresse Postale		Post Office Address	
Nom complete du cinquième co-inventeur, le cas échéant		Full name of fifth joint inventor, if any	
Signature de l'inventeur	Date	Fifth inventor's signature	Date
Domicile		Residence	
Nationalité		Citizenship	
Adresse Postale		Post Office Address	
Nom complete du sixième co-inventeur, le cas échéant		Full name of sixth joint inventor, if any	
Signature de l'inventeur	Date	Sixth inventor's signature	Date
Domicile		Residence	
Nationalité		Citizenship	
Adresse Postale		Post Office Address	

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

SEQUENCE LISTING

<110> KONDOROSI, Eva
CEBOLLA, Angel
KONDOROSI, Adam

<120> PLANT PROTEIN WITH REPEATED WD40 MOTIFFS, NUCLEIC ACID CODING FOR
SAID PROTEIN, AND USES THEREOF

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Arg Asn Ile Phe Arg Tyr Lys Thr Glu Thr Arg Gln Ser Met His Ser
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Asp Asp Cys Val Cys Ser Val Gly Trp Ala Gln Arg Gly Thr His Leu
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Cys Lys Lys Ile Arg Ser Met Glu Gly His Arg Leu Arg Val Gly Ala
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Ile Tyr Gln Arg Asp Ile Arg Thr Gln Glu Asp Phe Val Ser Lys Leu
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Asn Ser His Leu Ser Cys Met Asp Thr Gly Ser Gln Val Cys Asn Leu
370 375 380

Val Trp Ser Lys Asn Val Asn Glu Leu Val Ser Thr His Gly Tyr Ser
 385 390 395 400

Gln Asn Gln Ile Ile Val Trp Arg Tyr Pro Thr Met Ser Lys Leu Ala
 405 410 415

Thr Leu Thr Gly His Thr Tyr Arg Val Leu Tyr Leu Ala Ile Ser Pro
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Asp Gly Gln Thr Ile Val Thr Gly Ala Gly Asp Glu Thr Leu Arg Phe
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 35 40 45

Ser Tyr Asn Thr Ser Phe Ser Gly Val Gln Ala Pro Thr Lys Thr Pro
 50 55 60

Gly Lys Ser Ser Glu Gly Lys Thr Lys Lys Ser Asn Thr Thr Pro Ser
 65 70 75 80

Lys Thr Pro Gly Gly Gly Asp Arg Phe Ile Pro Asn Arg Ala Ala Thr
 85 90 95

Asn Phe Glu Leu Ala His Phe Leu Val Asn Lys Asp Ser Gly Asp Lys
 100 105 110

Ser Asp Glu Glu Asn Asp Lys Ala Thr Ser Ser Asn Ser Asn Glu Ser
 115 120 125

Asn Val Gln Ala Ser Ala His Lys Gly Asp Arg Gln Lys Leu Ile Ser
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Glu Val Ala Gln Val Gly Asp Ser Lys Gly Gly Arg Ile Leu Cys Tyr
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Gln Asn Lys Ala Pro Ala Ala Pro Glu Thr His Asn Asn Pro Leu Lys
 165 170 175

Val Val Tyr Ser Ile Lys Thr Pro Ile Ser Thr Lys Ser Gly Ser Arg
 180 185 190

Tyr Ile Pro Thr Thr Ser Glu Arg Ile Leu Asp Ala Pro Asp Phe Ile

195

200

205

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Ala Val Ala Leu Gly Ser Cys Val Tyr Leu Trp Asn Ala Gln Thr Gly
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Asn Ile Glu Gln Leu Thr Glu Phe Glu Glu Gly Asp Tyr Ala Gly Ser
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Leu Ser Trp Ile Gln Glu Gly Gln Ile Leu Ala Ile Gly Asn Ser Thr
 260 265 270

Gly Ala Val Glu Leu Trp Asp Cys Ser Lys Val Lys Arg Leu Arg Val
 275 280 285

Met Asp Gly His Ser Ala Arg Val Gly Ser Leu Ala Trp Asn Ser Phe
 290 295 300

Leu Val Ser Ser Gly Ser Arg Asp Gly Thr Ile Val His His Asp Val
 305 310 315 320

Arg Ala Arg Glu His Lys Leu Ser Thr Leu Ser Gly His Thr Gln Glu
 325 330 335

Val Cys Gly Leu Lys Trp Ser Thr Asp Phe Lys Tyr Leu Ala Ser Gly
 340 345 350

Gly Asn Asp Asn Leu Val Asn Val Trp Ser Ala Ala Ser Gly Gly Val
 355 360 365

Gly Thr Ala Thr Asp Pro Leu His Lys Phe Asn Asp His Gln Ala Ala
 370 375 380

Val Arg Ala Leu Ala Trp Cys Pro Trp Gln Pro Ser Thr Leu Ala Ser
 385 390 395 400

Gly Gly Gly Thr Ala Asp Arg Cys Ile Lys Phe Trp Asn Val Asn Asn

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Gly Thr Leu Met Lys Ser Val Asp Ser Lys Ser Gln Val Cys Ser Leu
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Asn Asn Gln Leu Thr Ile Trp Lys Tyr Pro Thr Met Val Lys Gln Ala
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Asp Leu Thr Gly His Thr Ser Arg Val Leu Gln Met Ala Met Ser Pro
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Asp Gly Ser Thr Val Ile Ser Ala Gly Ala Asp Glu Thr Leu Arg Leu
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Ser Cys Leu Leu Lys Asn Glu Leu Leu Gly Ser Ala Ile Asp Asp Val
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Lys Thr Ala Gly Glu Glu Arg Asn Glu Asn Ala Tyr Thr Pro Ala Ala
100 105 110

Lys Arg Ser Leu Phe Lys Tyr Gln Ser Pro Thr Lys Gln Asp Tyr Asn
115 120 125

Gly Glu Cys Pro Tyr Ser Leu Ser Pro Val Ser Ala Lys Ser Gln Lys
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Phe Lys Val Leu Asp Ala Pro Glu Leu Gln Asp Asp Phe Tyr Leu Asn
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Leu Val Asp Trp Ser Ser Gln Asn Val Leu Ala Val Gly Leu Gly Ser
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Cys Val Tyr Leu Trp Ser Ala Cys Thr Ser Gln Val Thr Arg Leu Cys
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Asp Leu Ser Pro Asp Ala Asn Thr Val Thr Ser Val Ser Trp Asn Glu
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Gln Ser Glu Arg Arg Leu Ala Gly His Arg Gln Glu Val Cys Gly Leu
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Lys Trp Ser Pro Asp Asn Gln Tyr Leu Ala Ser Gly Gly Asn Asp Asn
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Arg Leu Tyr Val Trp Asn Gln His Ser Val Asn Pro Val Gln Ser Tyr
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Thr Glu His Met Ala Ala Val Lys Ala Ile Ala Trp Ser Pro His His
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Ser Gln Val Cys Asn Leu Ala Trp Ser Lys His Ser Ser Glu Leu Val
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Ser Thr His Gly Tyr Ser Gln Asn Gln Ile Leu Val Trp Lys Tyr Pro
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Ser Leu Thr Gln Val Ala Lys Leu Thr Gly His Ser Tyr Arg Val Leu
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Tyr Leu Ala Leu Ser Pro Asp Gly Glu Ala Ile Val Thr Gly Ala Gly
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Asp Glu Thr Leu Arg Phe Trp Asn Val Phe Ser Lys Ala Arg Ser Gln

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 35 40 45

Pro Ser Thr Val Tyr Gln Asp Arg Tyr Tyr Pro Ser Arg Thr Asp Ile
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Asp Phe Phe Ser Ile Val Ser Ile Ser Ser Met Ala Ser Val Pro Ala
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Leu Asn Pro Ser Ser Thr Lys Asp Gln Val Glu Tyr Gln Lys Lys Arg
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Gln Ala His Glu Thr Tyr Asn Thr Leu Leu Lys Asn Glu Leu Phe Gly
 100 105 110

Lys His Leu Ser Lys Asp Thr Val Gln Ser Lys Ser Ser Ile Asp Arg
 115 120 125

Ile Lys Asn Thr Arg Pro Ser Thr Arg Gln Asn Val His Ala Lys Asn

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135

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Lys Ala Ala Gly Leu Lys Lys Phe Ser Pro His Ser Thr Phe Val Thr
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Ala Pro Ser Leu Ala Asp Asp Phe Tyr Tyr Ser Leu Ile Asp Trp Ser
 260 265 270

Ser Thr Asp Val Leu Ala Val Ala Leu Gly Lys Ser Ile Phe Leu Thr
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Tyr Thr Ser Leu Ser Trp Ile Gln Ala Gly Ser His Leu Ala Val Gly
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Gln Ala Asn Gly Leu Val Glu Ile Tyr Asp Asp Val Met Lys Arg Lys
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Cys Tyr Arg Thr Leu Ser Gly His Ile Asp Arg Val Ala Cys Leu Ser

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345

350

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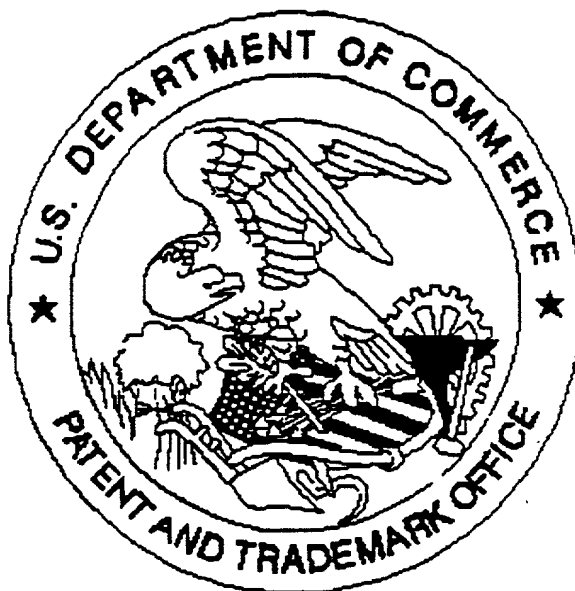
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